ORGANIC LETTERS

1999
Vol. 1, No. 1
99−102

Synthesis of a Reaction Intermediate Analogue of Biotin-Dependent Carboxylases via a Selective Derivatization of Biotin

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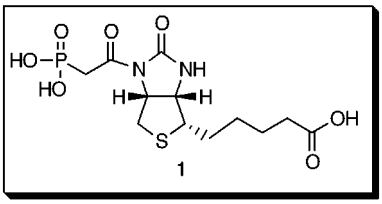
Received April 9, 1999

ABSTRACT

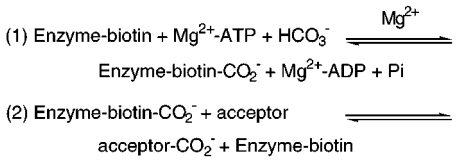


An efficient and practical synthesis of 1, a unique reaction intermediate analogue of biotin-dependent carboxylases, is described. The synthesis features a selective acylation of the 1′-N of biotin. Target 1 inhibits the activity of the biotin carboxylase component of acetyl CoA carboxylase. It is the first known biotin-derived inhibitor of biotin carboxylase and should promote new kinetic and structural studies of the biotin-dependent carboxylases.

Biotin functions as a cofactor for a group of enzymes that catalyze carboxylation reactions, transcarboxylation reactions, and decarboxylation reactions.[1,2] Currently, we are studying the kinetic and structural aspects of the biotin-dependent enzyme acetyl CoA carboxylase, which catalyzes the first step in fatty acid biosynthesis. These studies have been hampered by the low affinity (K_m = 134 mM) that biotin has for the enzyme.[3] One way to increase the affinity of biotin for acetyl CoA carboxylase might be to develop stable substrate analogues that incorporate biotin. To this end, we describe the synthesis and bioactivity of a unique first-generation biotin-derived inhibitor (**1**) of acetyl CoA carboxylase. The synthesis of **1** should allow for a variety of novel structural modifications of biotin.

All biotin-dependent carboxylases catalyze their respective reactions via the two-step sequence shown in Scheme 1.[2]

Scheme 1



The elegant studies of Lane and co-workers showed that in the first partial reaction biotin is carboxylated on the 1′-N.[4] Since bicarbonate is the source of CO_2 for all biotin-dependent carboxylases, the carboxylation of the 1′-N of

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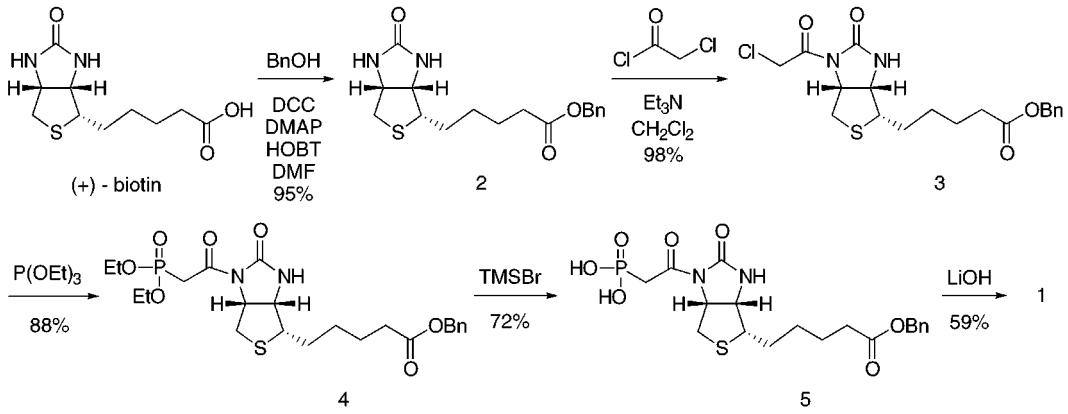
10.1021/ol990026z CCC: $18.00
Published on Web 05/17/1999

Scheme 2 Preparation of **1** from (+)-Biotin



biotin is accomplished by activating bicarbonate through phosphorylation with ATP to form a carboxyphosphate intermediate.[5] The carboxyl group is then transferred from carboxyphosphate to biotin to form carboxybiotin.

Target compound **1** (Figure 1) is formally derived from phosphonacetic acid coupled to the 1′-N of biotin. Thus, compound **1** should embody a stable analogue of the naturally occurring carboxyphosphate intermediate involved in biotin-mediated CO_2 transfer.

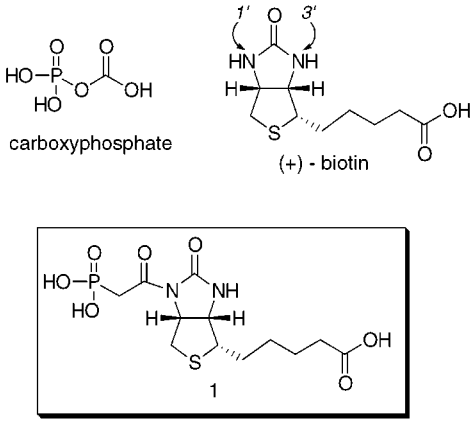


Figure 1. Carboxyphosphate, biotin, and target stable reaction intermediate analogue **1**.

In the area of biotechnology, biotin has become an essential reagent in methods used to label, detect, and purify proteins and nucleic acids.[6] These methods are based on the remarkable affinity biotin has for the proteins avidin and streptavidin. The dissociation constant of biotin from avidin and streptavidin is about 10^{-15} M, which is one of the tightest interactions between a protein and a ligand.[7] The hydrogen bonding of the ureido moiety is the major contributor to this tight binding.[7,8]

Given the fact that the ureido ring of biotin is of great importance for binding to avidin and that the 1′-N is directly involved in biotin's role as a carboxy transfer intermediate, it is surprising that relatively few studies have involved functionalization of the 1′-N of biotin.[9,10] The synthesis of **1** (Scheme 2) begins with protection of (+)-biotin as the corresponding benzyl ester in 95% yield via stirring DMF (60 mL), biotin (10.2 g, 42.6 mmol, 1 equiv), benzyl alcohol (5.6 g, 52 mmol, 1.2 equiv), DCC (46.2 mL of a 1 M solution in CH_2Cl_2, 46.2 mmol, 1.1 equiv), DMAP (0.51 g, 4.2 mmol, 0.1 equiv), and HOBt (0.64 g, 4.2 mmol, 0.1 equiv), at room temperature for 12 h.[11] Chloride **3** is obtained in 98% yield by careful dropwise addition of Et_3N (7.06 mL, 5.1 mmol, 3 equiv) and chloroacetyl chloride (1.95 mL, 25 mmol, 1.5 equiv) in three portions to a solution of biotin benzyl ester (5.6 g, 17 mmol, 1 equiv) in CH_2Cl_2 at −78 °C and warming to room temperature (12 h).[12] No diacylated product is observed under these conditions. Up to 25% diacylated product has been observed upon acylation of biotin methyl ester with methyl chloroformate[9] or trifluoroacetic anhydride.[10] We obtain up to 10% diacylation with biotin methyl ester and chloroacetyl chloride, as evidenced by ^{1}H NMR.

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(11) **2**: ^{1}H NMR (DMSO-d_6) δ 7.36 (s, 5H), 6.43 (s, 1H), 6.36 (s, 1H), 5.08 (s, 2H), 4.29 (m, 1H), 4.13 (m, 1H), 3.07 (m, 1H), 2.84−2.50 (m, 2H), 2.36 (t, J = 7.2 Hz, 2H), 1.65−1.25 (m, 6H); ^{13}C (DMSO-d_6) δ 173.59, 163.56, 137.15, 129.30, 128.79, 66.18, 61.88, 60.03, 56.20, 34.15, 28.84, 25.37; IR (KBr) 3244, 2930, 1740, 1692, 1458, 1428, 1262, 1170, 737, 698 cm^{-1}; HRMS *m*/*z* found 335.1432 (calcd for $C_{17}H_{23}N_2O_3S$, 335.1429 MH$^+$).

(12) **3**: ^{1}H NMR (DMSO-d_6) δ 8.11 (s, 1H), 7.36 (s, 5H), 5.08 (s, 2H), 4.81 (m, 3H), 4.19 (m, 1H), 3.20 (m, 1H), 3.03−2.83 (m, 2H), 2.37 (t, J = 7.2 Hz, 2H), 1.71−1.23 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 173.56, 166.32, 156.64, 137.14, 129.14, 128.80, 66.20, 62.20, 58.84, 55.42, 44.89, 38.03, 34.10, 28.81, 25.24; IR (thin film) 3332, 3134, 3134, 2937, 1735, 1704, 1395, 1358, 1241, 792, 752, 699 cm^{-1}; HRMS *m*/*z* found 411.1137 (calcd for $C_{19}H_{23}ClO_4N_2S$, 411.1245 MH$^+$).

Energy minimization studies (Sybil 6.1) show that a benzyl moiety blocks the 3′-N more effectively than does a methyl. Compound **3** (6.9 g, 17 mmol, 1 equiv) undergoes an Arbuzov reaction at 100 °C with $P(OEt)_3$ (20 mL, neat) to afford the phosphonate ester **4** in 89% yield.[13] The structure of **4** is confirmed by X-ray crystallography.[14] Phosphonate ester hydrolysis of **4** (4.75 g, 9.3 mmol, 1 equiv) promoted by TMSBr (3.7 mL, 28 mmol, 3 equiv) affords 3.0 g (72%) of phosphonic acid.[15] Subsequent saponification of **5** (0.51 g, 1.1 mmol, 1 equiv) with LiOH (0.21 g, 4.9 mmol, 4.5 equiv) affords the water-soluble target **1** (59%) for inhibition studies.[16]

If **1** is in fact a reaction intermediate analogue of biotin-dependent carboxylases, then it should act as an inhibitor. To test this hypothesis, the effect of **1** on the activity of biotin carboxylase from *Escherichia coli* was examined. Biotin carboxylase is one component of *E. coli* acetyl CoA carboxylase and catalyzes the first partial reaction shown in Scheme 1.[17] We chose it as a model for biotin-dependent carboxylases because the gene for the enzyme has been cloned and overexpressed.[18,19] In addition, the crystal structure of biotin carboxylase has been determined, which is the first and only three-dimensional model of a biotin-dependent carboxylase.[20]

Compound **1** does inhibit the activity of biotin carboxylase. Shown in Figure 2 are progress curves where the activity of biotin carboxylase is measured in the absence and presence of increasing amounts of **1**. As the concentration of **1** increases, the initial velocity of biotin carboxylase decreases.

Compound **1** was found to exhibit linear competitive inhibition with respect to the substrate ATP. Fitting the data to the equation for linear competitive inhibition by nonlinear regression analysis yielded a slope inhibition constant of 8.4 ± 1.1 mM. While this is a modest degree of inhibition, compared to the K_m for biotin in biotin carboxylase (134 mM), simply by positioning a phosphonacetyl moiety on the 1′-N of biotin, the affinity of biotin for biotin carboxylase increases dramatically. Moreover, a previous study found that none of the current biotin derivatives inhibited biotin carboxylase;[21] therefore, **1** represents the first biotin-derived inhibitor of biotin carboxylase.

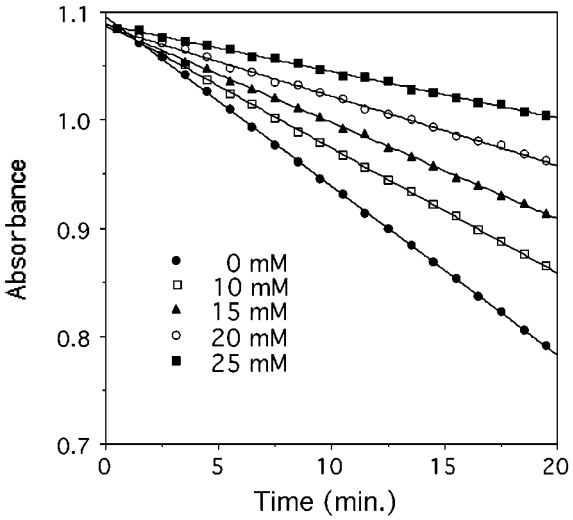

Figure 2. Progress curves for biotin carboxylase activity with increasing amounts of **1**. The activity of biotin carboxylase was measured by following the production of ADP using pyruvate kinase and lactate dehydrogenase. The oxidation of NADH by lactate dehydrogenase at 340 nm is measured with respect to time. The substrate concentrations were held constant at 9 mM for bicarbonate, 0.1 mM for ATP, and 100 mM for biotin.

In conclusion, we have developed an efficient route toward **1,** a reaction intermediate analogue of a biotin-dependent carboxylase, based on the reaction intermediate carboxyphosphate. Compounds **1** and **3** embody a versatile new biotin-based substrate and electrophilic coupling template, respectively, that should promote ready access to new families of active biotin materials. Currently we are preparing and testing a variety of congeners of **1** as inhibitors of biotin carboxylase. These compounds could serve as leads for novel antihyperlipidemic drugs and highly specific biodegradable herbicides, on the basis of biotin carboxylase's role in fatty acid biosynthesis.

Acknowledgment. We thank Dr. W. Dale Treleaven for assistance in obtaining NMR spectra. This research was supported by a grant from the NIH (Grant No. GM51261) to G.L.W. The latter stages of this work were supported by the Petroleum Research Fund of the American Chemical Society (Grant No. 32234-AC4) to G.L.W. Support is also acknowledged from Louisiana State University. Mass spectrometry was provided by the Washington University Mass Spectrometry Resource, an NIH Research Resource (Grant

(13) **4**: ^{1}H NMR ($CDCl_3$) δ 7.28 (s, 5H), 5.04 (s, 2H), 4.85 (t, 1H), 4.09 (q, J = 6.9 Hz, 4H), 3.78 (m, 2H), 3.09 (m, 1H), 2.94 (m, 1H), 2.29 (t, J = 7.2 Hz, 2H), 1.74−1.36 (m, 6H), 1.28 (t, J = 6.9 Hz, 6H); ^{31}P NMR (DMSO-d_6) δ 21.3, (s); ^{13}C NMR (DMSO-d_6) δ 173.56, 165.02, 157.51, 156.60, 137.14, 129.30, 128.79, 66.20, 62.57, 62.11, 60.62, 57.68, 55.46, 38.29, 34.64, 34.108, 32.92, 28.81, 28.58, 25.24; IR (thin film) 3328, 3262, 2981, 2935, 2867, 1737, 1678, 1396, 1351, 1251, 1149, 1025, 753, 699 cm^{-1}; HRMS m/z found 513.1828 (calcd for $C_{23}H_{33}N_2O_7PS$, 513.1824 MH^+).

(14) Crystal data for **4**: $C_{23}H_{33}N_2O_7PS$, monoclinic space group $P2_1$, a = 10.546(1) Å, b = 12.550(1) Å, c = 10.674(1) Å, β = 112.15(1)°, V = 1308.4(5) Å^3, Z = 2, colorless, T = 299 K, R = 0.096, GOF = 4.753. Displacement parameters of the benzyl ester and phosphonate groups are large; low-temperature data collection is planned.

(15) **5**: ^{1}H NMR (DMSO-d_6) δ 7.96 (s, 1H), 7.36 (s, 5H), 5.1 (s, 2H), 4.77 (t, 1H), 4.1 (m, 1H), 3.5 (m, 2H), 3.2 (m, 2H), 3.07−2.80 (m, 2H), 2.37 (t, J = 7.2 Hz, 2H), 1.69−1.36 (m, 6H); ^{31}P NMR (DMSO-d_6) δ 15.5 (s); ^{13}C NMR (DMSO-d_6) δ 173.57, 166.40, 157.48, 156.61, 129.31, 128.81. 66.20, 62.10, 57.64, 55.40, 38.47, 35.44, 34.11, 28.82, 28.60, 25.25; IR (KBr) 3434, 2935, 2856, 1737, 1682, 1399, 1356, 1233, 1184, 1149, 753, 698 cm^{-1}; HRMS m/z found 457.1194 (calcd for $C_{19}H_{25}N_2O_7PS$, 457.1198 MH^+).

(16) **1**: ^{1}H NMR (D_2O) δ 4.50 (m, 1H), 4.34 (m, 1H), 3.25 (m, 1H), 2.92−2.64 (m, 2H), 2.43−2.36 (m, 2H), 2.06 (t, J = 7.2 Hz, 2H). 1.69−1.25 (m, 6H); ^{31}P NMR (D_2O) δ 14.9 (s); ^{13}C NMR (D_2O) δ 157.49, 62.33, 60.59, 55.69, 41.38, 40.05, 39.86, 37.65, 28.59, 28.00, 25.00; IR (KBr) 3344, 3196, 2922, 2851, 1702, 1661, 1567, 1431, 1138, 1074, 869 cm^{-1}; MS m/z found 394.4 (calcd for $C_{12}H_{17}LiN_2NaO_7PS$, 394.0552 MH^+).

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No. P41RR0954), and the Nebraska Center for Mass Spectrometry.

Supporting Information Available: Figures giving NMR spectra, text giving procedures for the preparation of compounds **1**−**5**, and X-ray structural information for **4**, including an X-ray crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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